

What is claimed is:

1. A method for faithfully amplifying a complex population of mRNA molecules, said method comprising:

(a) generating double-stranded cDNA molecules from a population of RNA molecules, wherein a first-strand synthesis reaction for generating said cDNA molecules comprises a primer complementary to the mRNA molecules; and

(b) *in vitro* transcribing the cDNA molecules of step (a) to generate amplified antisense RNA molecules,

wherein prior to or during said *in vitro* transcription, the synthesis of template-independent product is reduced.

2. A method for amplifying a complex population of mRNA molecules, said method comprising:

(a) generating cDNA from a population of template RNA molecules, wherein a first-strand synthesis reaction comprises a primer complementary to the mRNA molecules; and

(b) *in vitro* transcribing the cDNA molecules of step (a) to generate amplified RNA molecules,

wherein the primer of step (a) is present at a concentration of no greater than 0.2  $\mu\text{M}$  in step (b).

3. The method of claim 2, wherein the primer from step (a) is present at a concentration of no greater than 0.02  $\mu$ M in step (b).
4. The method of claim 2, wherein the primer is present at a concentration of no greater than 1  $\mu$ M in step (a).
5. The method of claim 2, wherein the mRNA molecules are amplified from no more than 10  $\mu$ g of total RNA.
6. The method of claim 2, wherein the mRNA molecules are amplified from no more than 1  $\mu$ g of total RNA.
7. The method of claim 2, wherein the mRNA molecules are amplified from no more than 100 ng of total RNA.
8. The method of claim 2, wherein the mRNA molecules are amplified from no more than 10 ng of total RNA.
9. The method of claim 2, wherein the mRNA molecules are amplified from no more than 2 ng of total RNA.
10. The method of claim 2, wherein the mRNA molecules are amplified from total RNA isolated from fewer than 1000 cells.
11. The method of claim 2, wherein the mRNA molecules are amplified from total RNA isolated from fewer than 100 cells.
12. The method of claim 2, wherein the mRNA molecules are amplified from total RNA isolated from fewer than 10 cells.

13. The method of claim 2, wherein the mRNA molecules are amplified from total RNA isolated from a single cell.
14. The method of claim 2, wherein the primer comprises a polythymidine sequence.
15. The method of claim 2, wherein the primer comprises a promoter sequence for an RNA polymerase.
16. The method of claim 15, wherein the promoter sequence is from bacteriophage T7.
17. The method of claim 2, further comprising the steps of:
- (c) generating double-stranded cDNA molecules from the antisense molecules of step (b); and
  - (d) *in vitro* transcribing the cDNA molecules of step (c) to generate amplified antisense RNA molecules.

18. A method for amplifying a complex population of mRNA molecules, said method comprising:

(a) generating double-stranded cDNA molecules from a population of mRNA molecules, wherein a first-strand synthesis reaction for generating said cDNA molecules comprises a single-strand binding protein at a concentration sufficient to support completed synthesis of templates longer than 600 nucleotides in length by a reverse transcriptase and a primer complementary to the mRNA molecules; and

(b) *in vitro* transcribing the cDNA molecules of step (a) to generate amplified antisense RNA molecules,

wherein the primer from step (a) is present at a concentration of no greater than 0.2  $\mu$ M in step (b).

19. The method of claim 18, wherein the primer from step (a) is present at a concentration of no greater than 0.02  $\mu$ M in step (b).

20. The method of claim 18, wherein the single-strand binding protein is present at a concentration of at least 0.015 mM.

21. The method of claim 18, wherein the single-strand binding protein is present at a concentration of at least 0.0061 mM.

22. The method of claim 18, wherein the single-strand binding protein comprises T4 gp32.

23. The method of claim 18, wherein the single-strand binding protein comprises the single-strand binding protein of *Escherichia coli*.

24. The method of claim 18, wherein the primer is present at a concentration of no greater than 1  $\mu$ M in step (a).

25. The method of claim 18, wherein the mRNA molecules are amplified from no more than 10  $\mu$ g of total RNA.

26. The method of claim 18, wherein the mRNA molecules are amplified from no more than 1  $\mu$ g of total RNA.

27. The method of claim 18, wherein the mRNA molecules are amplified from no more than 100 ng of total RNA.

28. The method of claim 18, wherein the mRNA molecules are amplified from no more than 10 ng of total RNA.

29. The method of claim 18, wherein the mRNA molecules are amplified from no more than 2 ng of total RNA.

30. The method of claim 18, wherein the mRNA molecules are amplified from total RNA isolated from fewer than 1000 cells.

31. The method of claim 18, wherein the mRNA molecules are amplified from total RNA isolated from fewer than 100 cells.

32. The method of claim 18, wherein the mRNA molecules are amplified from total RNA isolated from fewer than 10 cells.

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37. A method for amplifying a complex population of RNA molecules from less than 100 ng of total RNA, said method comprising:

(a) generating double-stranded cDNA molecules from a population of RNA molecules, wherein a first-strand synthesis reaction for generating said cDNA molecules comprises a primer complementary to the mRNA molecules and a single-strand binding protein at a concentration sufficient to support completed synthesis of RNA templates greater than 600 nucleotides in length by a reverse transcriptase; and

(b) *in vitro* transcribing the cDNA molecules of step (a) to generate amplified RNA molecules; and

(c) generating double-stranded cDNA molecules from the antisense RNA molecules of step (b), wherein a first-strand synthesis reaction for generating said cDNA molecules comprises a single-strand binding protein at a concentration sufficient to support completed synthesis of RNA templates greater than 600 nucleotides in length by a reverse transcriptase; and

(d) *in vitro* transcribing the cDNA molecules of step (c) to generate amplified RNA molecules,

wherein the primer from step (a) is present at a concentration of no greater than 0.2  $\mu$ M in step (b).

38. The method of claim 37, wherein the primer from step (a) is present at a concentration of no greater than 0.02  $\mu$ M in step (b).

39. The method of claim 37, wherein the single-strand binding protein is present at a concentration of at least 0.015 mM.

40. The method of claim 37, wherein the single-strand binding protein is present at a concentration of at least 0.0061 mM.

41. The method of claim 37, wherein the single-strand binding protein comprises T4 gp32.

42. The method of claim 37, wherein the single-strand binding protein comprises the single-strand binding protein of *Escherichia coli*.

43. The method of claim 37, wherein the primer is present at a concentration of no greater than 1  $\mu$ M in step (a).

44. The method of claim 37, wherein the mRNA molecules are amplified from no more than 10 ng of total RNA.

45. The method of claim 37, wherein the mRNA molecules are amplified from no more than 2 ng of total RNA.

46. The method of claim 37, wherein the mRNA molecules are amplified from total RNA isolated from fewer than 1000 cells.

47. The method of claim 37, wherein the mRNA molecules are amplified from total RNA isolated from fewer than 100 cells.

48. The method of claim 37, wherein the mRNA molecules are amplified from total RNA isolated from fewer than 10 cells.



49. The method of claim 37, wherein the mRNA molecules are amplified from total RNA isolated from a single cell.

50. The method of claim 37, wherein the primer comprises a polythymidine sequence.

51. The method of claim 37, wherein the primer comprises a promoter sequence for an RNA polymerase.

52. The method of claim 51, wherein the promoter sequence is from bacteriophage T7.

53. A method for synthesizing cDNA molecules, said method comprising a reverse transcriptase and a single-strand binding protein at a concentration sufficient to promote completed reverse transcription of mRNA molecules greater than 600 nucleotides in length.

54. The method of claim 53, wherein the single-strand binding protein is present at a concentration of at least 0.0061 mM.

55. The method of claim 53, wherein the single-strand binding protein is present at a concentration of at least 0.015 mM.

56. The method of claim 53, wherein the single-strand binding protein comprises T4 gp32.

57. The method of claim 53, wherein the single-strand binding protein comprises the single strand binding protein of *Escherichia coli*.

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58. The method of claim 53, wherein the cDNA synthesis reaction is carried out at a temperature of no more than 42 degrees celsius.

59. A kit comprising in one or more containers:

(a) a primer comprising a sequence complementary to mRNA molecules for first-strand cDNA synthesis; and

(b) a reverse transcriptase; and

(c) a single-strand binding protein at a concentration of at least 0.25 mM.

60. The kit of claim 59, further comprising instructions for its use.

61. The kit of claim 59, further comprising instructions for limiting the synthesis of template-independent products.

62. The kit of claim 59, wherein the single-strand binding protein comprises T4 gp32.

63. The kit of claim 59, wherein the single-strand binding protein comprises the single-strand binding protein of *Escherichia coli*.

64. The kit of claim 59, further comprising:

(d) instructions for increasing the processivity of the RNA polymerase; and

(e) a set of control RNA molecules of several different and known lengths, suitable for the measurement of completed synthesis of mRNA transcripts longer than 600 nucleotides.

65. The kit of claim 64, wherein the control RNA molecules include RNA molecules greater than 4000 nucleotides in length.

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